REMARKS

Claims 1, 3, 11, and 14, as herein amended, claims 13 and 16 as filed, and new claims 29-35 are pending. Claims 5-10, 13 and 17-22 have been withdrawn from consideration without prejudice or disclaimer in response to the restriction requirement, and claims 2, 4, 12 and 15 have been cancelled without prejudice. Applicant wishes to retain his rejoinder rights to all claims capable of rejoinder, and elect to defer making any required amendments until such time as the pending claims are acknowledged to be patentable.

All grounds of rejection to any cancelled claim are not addressed in this response, as Applicant's cancellation thereof has rendered these rejections moot.

The claims, as amended, fulfill the requirements of 35 U.S.C. §112.

Claims 14 and 16 stand rejected under 35 U.S.C. §112, first paragraph for failing to satisfy the written description requirement. Specifically, the Action asserts that Applicant's previously-submitted amendment, "detecting response to said therapy when a decreased amount of her-2/neu RNA is detected," is not supported by the specification as filed and thus constitute new matter.

Without acquiescing to the correctness of this assertion, Applicant has amended the pending claims to delete the objected-to amendment. Applicant thus respectfully contends that these amendments have overcome the asserted ground of rejection and request that the Examiner withdraw these grounds of rejection.

Claims 1, 3, 11 and 13 stand rejected under 35 U.S.C. §112, first paragraph for failing to satisfy the enablement requirement. The Action acknowledges that the claims are enabled for detecting her2/neu in blood plasma or serum in patients having certain cancers, it asserts that the claims as filed are not enabled throughout their full scope.

Without acquiescing to the asserted grounds of rejection, Applicant has amended his pending claims to recite that his invention is directed to detecting certain species of RNA, including epidermal growth factor receptor RNA, her-2/neu RNA, and heterogeneous nuclear ribonucleoprotein A2/B1 RNA in human blood plasma or serum from humans with certain cancers. Applicant's amendment thus addresses the issues raised in the justification for the rejection contained in the Action. Specifically, these amendments are submitted to overcome the assertion in the Action that claim 1 is not enabled for detected "two or more" RNA species, or

that claim 3 is not enabled for detecting her-2/neu, or that claims 11 and 13 are not enabled for evaluating a human having "any kind of cancer." Thus, claim 1 has been amended to be directed to detecting epidermal growth factor receptor RNA in blood plasma or serum from a human having colorectal cancer. Claim 3 has been amended to directed to detecting her-2/neu RNA in blood plasma or serum from a human having breast cancer. Also, the amendments have overcome the objection that the probe can encompass poly(T) because RNA species comprise a poly(A) tail sequence. Claim 11 has been amended to be directed to detecting her-2/neu RNA in blood plasma or serum from a human having breast cancer.

Applicant respectfully contends that these amendments address the bases asserted in the Action in support of the determination that the claims prior to amendment were not enabled. Applicant respectfully disagrees with the assertions contained in the Action, but has amended the claims as set forth herein in the interest of expediting examination of the pending claims to allowance.

All pending claims are further rejected under 35 U.S.C. §112, second paragraph as being indefinite for reciting certain claim language. Applicant respectfully contends that the grounds of rejection asserted against Claim 1 has been overcome by the amendments discussed above with regard to the enablement rejections. Claim 3 is rejected because the Action asserts that the claim language is unclear regarding the species hybridized using the claimed method. Applicants have amended Claim 3 to clarify this aspect of their invention, wherein "the method comprising the step of hybridizing a primer or probe specific for her-2/neu RNA obtained from blood plasma or serum of a human with breast cancer or by hybridizing a primer or probe specific for cDNA produced from her-2/neu RNA obtained from blood plasma or serum of a human with breast cancer." Claim 11 is rejected for failing to define a standard for evaluating a human with cancer for a her-2/neu-directed therapy. Applicant has amended Claim 11 to recite that a her-2/neu-directed therapy is administered to a human with cancer "when her-2/neu RNA or cDNA prepared therefrom is detected in blood plasma or serum." Claim 14 is rejected because the Action asserts it is unclear that the amount of her-2/neu RNA detected decreases in response to the therapy. This claim has been amended as discussed above with regard to rejections based on the written description requirement, and Applicant respectfully contends that these amendments overcome this ground of rejection. Applicant's amendments also overcome the other ground of rejection asserted in the Action against Claim 14, wherein the claim now

recites the proper antecedent basis for the phrase "a human with breast cancer receiving a her-2/neu directed therapy."

Applicant thus respectfully contends that these amendments overcome the asserted grounds of rejection, and request the Examiner to withdraw these rejections.

The claims are not anticipated by the cited reference.

Claim 1 stands rejected as being anticipated under 35 U.S.C. §102(b) by the teachings of the Balazs reference. The Action asserts that Balazs teaches detection of epidermal growth factor receptor RNA and c-myc by extracting total extracellular RNA from plasma or serum. Applicant's prior arguments with regard to the limitations of the teachings of the Balazs reference were deemed unpersuasive; Applicant thus submits amendments and argument to further distinguish his claims from the teachings of the reference.

With regard to Applicant's previous arguments regarding the Balazs teachings requiring the presence of a nuclease inhibitor (specifically, an RNase inhibitor) to be mixed with whole blood prior to separating plasma from the cellular fraction of the blood, Applicant respectfully contends that the Balazs reference cannot be properly understood without considering the teaching in Balazs that a nuclease is required. This is because the consequences of following the Balazs teachings impacts whether what Balazs and Applicant teach are in fact the same, *i.e.*, whether any RNA detected using the Balazs teachings is in fact "extracellular RNA" as that term is used in Applicant's specification. In view of what was know in the art at the time of the instant invention, it was well recognized that nucleases, specifically RNases, existed in blood plasma or serum, and it was expected that these enzymes would degrade any extracellular RNA that might otherwise be present in plasma or serum. Such RNases (ribonucleases) were expected to degrade the relatively fragile RNA within seconds. Moreover, RNases were reported to be elevated in the blood of cancer patients. This understanding was not changed even after disclosure of the Balazs reference. Specific reference to this understanding is included in the following prior art documents, of record in this application, including the Komeda (1995), Pfleiderer (1995), Ng et al. (2002) and Tsui et al. (2002) references previously provided. The Balazs reference is limited to detecting specific RNA species in the plasma or cancer patients only provided that a nuclease inhibitor is present in the preparation at all times. The reference provides no teaching that any such RNA detected in a plasma sample would be *extracellular*

RNA, *i.e.*, RNA present in the blood sample as extracellular RNA prior to any experimental manipulation of the sample. This is because the added nuclease inhibitor would be expected to protect from nuclease degradation any *intracellular* RNA released during sample preparation. This is particularly true when plasma is isolated from blood.

In keeping with the understanding of the art, the Balazs reference requires the inactivation of such ribonucleases by adding an RNase inhibitor prior to isolating plasma as a condition for detecting RNA from plasma. This requirement is explicitly stated in the reference:

- Balazs (WO), abstract: "under the constant effect of a reliable RNase inhibitor…."

- Balazs (WO), pg 4: "The RNase-handled sample of this same RNA plasma was ineffective."

- Balazs (WO), pg 13: "This task is accomplished by the process as described in Claim 1. The degradation of RNA or its fragments is prevented by the use of an effective and reliable RNase inhibitor that does not induce RNA exudation from the cells, where this inhibitor is used early during specimen collection of the cellular biologic liquid. The fact that the RNase inhibitor does not induce RNA exudation from the cells prior to and during their removal is significant, because this sensitive method can identify even small amounts of contamination." (emphasis added)

- Balazs (WO), pg 14: "The following describes the invention in more detail. The cellular biological liquid (such as blood, exudates, etc.) is mixed with a reliable RNase inhibitor that does not generate RNA cell leakage as early as during specimen collection, and the cells are removed. The total RNA of the resulting acellular biological liquid is mixed with a watery medium with continuous action of the RNase inhibitor …." (emphasis added)

- Balazs (WO), pg 16, Example: " 10 ml blood with 20 IE heparin are taken and immediately mixed with a solution of RNase inhibitors such as RNasin…. The blood plasma is separated as quickly as possible." (emphasis added)

In contrast, Kopreski teaches the presence of extracellular RNA in plasma and serum, irrespective of the addition of a RNase inhibitor. The methods taught in the instant specification do not require addition of an RNase inhibitor prior to separation of plasma. This is because the Applicant recognized, as Balazs and the rest of the art did not, that adding an RNase inhibitor prior to separating the cellular and acellular fractions of blood would stabilize any *intracellular*

RNA released from cells during the separation process, and thus provide contaminating intracellular RNA into the plasma sample. One of ordinary skill would recognize this deficiency in the method disclosed in the Balazs reference, and would understand that as a consequence Balazs does not teach a method that could be used to (unambiguously) detect extracellular RNA in blood plasma. The instant inventor found, surprisingly, that extracellular RNA is sufficiently stable even in the purported presence of serum RNases that it can be detected in human blood plasma or serum without adding RNase inhibitors, as evidenced by detection of extracellular RNA species using the methods disclosed in the instant specification. Adding RNases to blood prior to separating the cellular from the acellular portions thereof is thus not only unnecessary to stabilize extracellular RNA, but can stabilize any artifactually-produced *intracellular* RNA inadvertently released from blood cells during plasma sample separation. Although the Balazs reference recognizes that intracellular RNA contamination should be avoided, its own teachings subvert its intention to avoid detecting these artifactual intracellular RNA species.

This is a particularly-important consideration in methods directed towards detecting extracellular RNA in plasma or serum. As taught in the instant specification, plasma is produced by subjecting blood samples to centrifugation, a technique known to risk cell breakage and release of intracellular RNA species. Balazs requires that "cell leakage" must be avoided:

> "Through the use of an effective and reliable RNase inhibitor, but one that does not give rise to the escape of RNA from cells, even in sampling the cellular biological liquid, the degradation of the RNA or its fragments is prevented. Since the selected RNase inhibitor does not cause any escape of RNA from the cells before or during their removal, it is important because this sensitive method can already detect a small contamination."
>
> "The cellular biological fluid … is mixed with a reliable RNase inhibitor that does not give rise to RNA-cell leakage during sampling, and the cells are removed." (page 12, underlined for emphasis)

These passages illustrate that Balazs was concerned with the effects of added nuclease inhibitors in "giving rise" to intracellular RNA release; however, Balazs contains no teachings directed to preferred blood separation methods for preventing such release. As noted above, the art recognized that the presence of blood nucleases would be expected to degrade any *intracellular* RNA released from blood cells, but Balazs teaches to inhibit blood nucleases in a way that prevents degradation of any intracellular RNA released from cells during the separation of plasma from whole blood. This results in a substantial and unpredictable risk that the plasma

sample will be contaminated with intracellular RNA. While the Balazs reference recognizes the need to avoid "RNA-cell leakage" and "escape of RNA from cells," following those teachings would lead the skilled worker to avoid using separation methods likely to cause cell rupture or leakage, including centrifugation. In contrast, the instant invention teaches centrifugation as a method of separating plasma from a blood specimen.

Finally, there is no teaching in the Balazs reference for detecting extracellular RNA in serum, which is produced from blood by permitting the blood to clot. Moreover, under the circumstances of clotting it would be expected by the skilled worker that intracellular RNA would be released and stabilized by the introduction (as taught by Balazs) of RNases to whole blood. Thus, the application of the Balazs teachings to serum (not taught by Balazs) would produce a sample likely to be extensively contaminated by intracellularly-derived RNA species. Consequently, one skilled in the art would understand that Balazs does not teach or anticipate the detection of extracellular RNA from serum.

Additional distinctions are drawn by Applicant's instantly-amended claims, wherein the claims recite detecting extracellular RNA in patients with breast, colon and lung cancer. The Balazs reference does not disclose detecting RNA from blood for any of these cancers. To the contrary, Balazs states:

> "Because of the special properties of malignant cells and malignant tumor, the activated oncogene transcripts can get into the bloodstream…." (page 9).

The Balazs reference does not describe the nature of these "special properties" or if they are characteristic of all cancers. However, it is well understood that not all cancer types are characterized by the same properties. In particular, the Balazs reference does not teach that breast cancer, colorectal cancer, or lung cancer are characterized by these "special properties". In contrast, Kopreski teaches the presence of extracellular RNA in blood applicable to cancers including breast, colorectal, and lung, and further to premalignant conditions and normal cells.

Applicant respectfully contends that the Balazs reference thus does not teach detection of extracellular RNA of any species from blood plasma or serum, and for the reasons set forth above does not anticipate the claimed invention.

McDONNELL BOEHNEN HULBERT & BERGHOFF LLP
300 South Wacker Drive
Chicago, Illinois 60606
(312) 913-0001

The claims are non-obvious in view of the cited reference.

Claim 3 stands rejected under 35 U.S.C. 103 over the teachings of the Balazs reference taken in combination with the Revillion reference. Applicant respectfully traverses this ground of rejection.

The deficiencies of the Balazs reference are set forth above. In addition, in the context of non-obviousness the Balazs reference suffers from the following additional deficiencies. The reference does not negate the findings of the art as a whole (such as the Komeda and Pfleiderer reference) that extracellular RNA could not exist in blood plasma or serum under the constant presence of circulating ribonucleases. Instead, the Balazs reference suggests that ribonucleases would degrade any RNA found in plasma unless an RNase inhibitor was added prior to separating the cellular and acellular portions of blood. It would not be evident to the skilled worker how extracellular RNA, which the prior art teaches would not be expected to exist *in vivo* in the presence of circulating ribonucleases in blood, could be obtained from a blood specimen, other than as an artifact.

In contrast, Applicant's specification teaches that extracellular RNA can be detected in plasma without requiring nuclease inhibitors being added to whole blood prior to separation. This teaching of the instant specification was surprising and unexpected in view of the prior art as a whole, and specifically in view of the Balazs reference, since it was contrary to the notion that extracellular RNA was not detectable in blood plasma or serum due to the presence of blood ribonucleases expected to degrade any such RNA species. The understanding of the art, including the Balazs reference, is relevant because it motivated Balazs to introduce RNase inhibitors to blood prior to separating blood cells from plasma. As a consequence, following the teachings of the Balazs reference results in a plasma-RNAase inhibitor mixture wherein intracellular RNA released from blood cells "broken" during the separation process would have become detectable following the inactivation of naturally occurring RNases. Thus, the skilled worker would not have been in possession of a method to detect extracellular RNA species in blood plasma following the teachings of the Balazs reference.

The teachings of the Revillion reference do not overcome the deficiencies of the Balazs reference. Applicant argued previously that the Revillion reference was not prior art, because the Office did not recognize the instant application's priority to the filing date of U.S. provisional

application Serial No. 60/014,730. The Office's refusal was based on the grounds that U.S. provisional application Serial No. 60/014,730 does not teach detection of her-2/neu RNA species. To the contrary, Applicant respectfully contends that these teachings can be found on page 16, line 12, and example case 4, page 28, line 20 of the priority provisional application. Applicant thus respectfully contends that the priority date of the instant application is March 26, 1996, and therefore Revillion et al is not prior art.

Even taking the Revillion reference to be prior art to Applicant's invention, the reference merely teaches that the art recognized how to amplify her-2/neu. Applicant respectfully contends that the issue regarding claim 3 is whether the cited art would have made it obvious that extracellular her-2/neu RNA species could be detected in blood plasma or serum in a human with breast cancer. The teachings of the Revillion reference are not directly related to this issue.

Applicant thus respectfully contends that, even if the teachings of the Balazs reference are taken in combination with the Revillion reference his claimed invention is non-obvious, for the reasons set forth herein.

CONCLUSION

Applicant believes that all grounds of rejection have been overcome by amendment, and request that the pending claims be passed to issue.

If Examiner Lu believes it to be helpful, he is invited to contact the undersigned representative by telephone at (312) 913-0001.

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff LLP

Dated: July 28, 2008

By: /Kevin E. Noonan/
Kevin Noonan, Ph.D.
Reg. No. 35,303

McDONNELL BOEHNEN HULBERT & BERGHOFF LLP
300 South Wacker Drive
Chicago, Illinois 60606
(312) 913-0001